

Biological  
& Medical  
Series

*Imperial* **STORAGE** *Public*  
Agricultural Research Institute, Pusa

# A Note on the M'Fadyean Staining Reaction for Anthrax Bacilli

BY

MAJOR J. D. E. HOLMES, M.A., D.Sc., M.R.C.V.S.

*Imperial Bacteriologist, Muktesar Laboratory*



FEB 23 1914

CALCUTTA  
SUPERINTENDENT GOVERNMENT PRINTING, INDIA  
1913

Price 4 annas or 5 pence



# Publications of the Imperial Department of Agriculture in India.

To be obtained from :—

The Office of the Agricultural Adviser to the Government of India, Pusa, Bihar, and from the following Agents :—

1. Messrs. Thacker, Spink & Co., Calcutta.
2. Messrs. W. Newman & Co., Calcutta.
3. Messrs. Higginbotham, Ltd., Madras.
4. Messrs. D. B. Taraporevala, Sons & Co., Bombay.

**The Agricultural Journal of India.** [A Quarterly Journal dealing with subjects connected with agricultural economics, field and garden crops, economic plants and fruits, soils, manures, methods of cultivation, irrigation, climatic conditions, insect pests, fungus diseases, co-operative credit, agricultural cattle, farm implements and other agricultural matters in India. Illustrations, including coloured plates, form a prominent feature of the Journal. It is edited by the Agricultural Adviser to the Government of India assisted by an Advisory Committee of the staff of the Agricultural Research Institute, Pusa.]  
*Annual subscription*, Rs. 6 or Rs. 6d., including postage. Single copy, Rs. 2.

**Annual Report of the Imperial Department of Agriculture in India for the year 1904-05.** Price, As. 12 or 1s. 2d. (*Out of print.*)

**Report of the Imperial Department of Agriculture in India for the years 1905-06 and 1906-07.** Price, As. 6 or 7d.

**Report on the Progress of Agriculture in India for 1907-09.** Price, As. 6 or 7d.

**Report on the Progress of Agriculture in India for 1909-10.** Price, As. 6 or 7d.

**Report on the Progress of Agriculture in India for 1910-11.** Price, As. 12 or 1s. 3d.

**Report on the Progress of Agriculture in India for 1911-12.** Price As. 6 or 7d.

**Report of the Agricultural Research Institute and College, Pusa (including the Report of the Imperial Cotton Specialist), for 1907-09.** Price, As. 4.

**Report of the Agricultural Research Institute and College, Pusa (including the Report of the Imperial Cotton Specialist), for 1909-10.** Price, As. 4.

**Report of the Agricultural Research Institute and College, Pusa (including Report of the Imperial Cotton Specialist), for 1910-11.** Price, As. 6 or 7d.

**Report of the Agricultural Research Institute and College, Pusa (including the Report of the Imperial Cotton Specialist) for 1911-12.** Price As. 6 or 7d.

**Report of the Agricultural Research Institute and College, Pusa (including the Report of the Imperial Cotton Specialist, for 1912-13.** (*In the press.*)

**Proceedings of the Board of Agriculture in India held at Pusa on the 6th January 1905 and following days (with Appendices).** Price, As. 8 or 9d.

**Proceedings of the Board of Agriculture in India held at Pusa on the 15th January 1906 and following days (with Appendices).** Price, As. 12 or 1s. 2d.

**Proceedings of the Board of Agriculture in India held at Cawnpur on the 18th February 1907 and following days (with Appendices).** Price, Re. 1-2 or 1s. 6d.

**Proceedings of the Board of Agriculture in India held at Pusa on the 17th February 1908 and following days (with Appendices).** Price, As. 8 or 9d.

**Proceedings of the Board of Agriculture held at Nagpur on the 15th February 1909 and following days (with Appendices).** Price, As. 8 or 9d.

**Proceedings of the Board of Agriculture in India held at Pusa on the 21st February 1910 and following days (with Appendices).** Price, As. 8 or 9d.

**Proceedings of the Board of Agriculture in India held at Pusa on 20th November 1911 and following days (with Appendices).** Price, As. 10 or 1s.

**Standard Curriculum for Provincial Agricultural Colleges as recommended by the Board of Agriculture, 1908.** Price, As. 4 or 5d.

**Memoirs of the Department of Agriculture in India.** (These are issued from time to time as matter is available, in separate series, such as Chemistry, Botany, Entomology and the like.)

## BOTANICAL SERIES.

Vol. I, No. I.

**Studies in root Parasitism. The Haustorium of *Santalum album*—Part I—Early Stages** by C. A. BARBER, M.A., F.L.S. Price, Re. 1.  
**Part II—The Structure of the Mature Haustorium and the Inter-relations between Host and Parasite** by C. A. BARBER, M.A., F.L.S. Price, Rs. 3.



Agricultural Research Institute, Pusa

A Note on the M'Fadyean Staining  
Reaction for Anthrax Bacilli

BY

MAJOR J. D. E. HOLMES, M.A., D.Sc., M.R.C.V.S.

*Imperial Bacteriologist, Muktesar Laboratory*



# AGENTS FOR THE SALE OF BOOKS

PUBLISHED BY THE

## Superintendent Government Printing, India, Calcutta

### IN THE UNITED KINGDOM.

Constable & Co., 10, Orange Street, Leicester Square, London, W.C.

Kegan Paul, Trench Trübner & Co., 68-74, Carter Lane, E.C.

Bernard Quaritch, 11, Grafton Street, New Bond Street, London, W.

P. S. King & Son, 2 and 4, Great Smith Street, Westminster, London, S. W.

H. S. King & Co., 65, Cornhill, and 9, Pall Mall, London.

Grindlay & Co., 54, Parliament Street, London, S.W.

W. Thacker & Co., 2, Creed Lane, London, E.C.

Luzac & Co., 46, Great Russell Street, London, W.C.

B. H. Blackwell, 50 and 51, Broad Street, Oxford.

Deighton, Bell & Co., Cambridge.

Oliver & Boyd, Tweeddale Court, Edinburgh.

E. Ponsonby, Ltd., 116, Grafton Street, Dublin.

### ON THE CONTINENT.

Otto Harrassowitz, Leipzig.

R. Friedlander & Sohn, Berlin, W. N., Carlstrasse, 11.

Karl W. Hiersemann, Leipzig.

Ernest Leroux, 28, Rue Bonaparte, Paris.

Martinus Nijhoff, The Hague, Holland.

### IN INDIA AND CEYLON.

Thacker, Spink & Co., Calcutta and Simla.

Newman & Co., Calcutta.

R. Cambray & Co., Calcutta.

S. K. Lahiri & Co., Calcutta.

B. Banerjee & Co., Calcutta.

The Calcutta School Book and Useful Literature Society, 309, Bow Bazar Street, Calcutta.

Butterworth & Co. (India), Limited, Calcutta.

The Weldon Library, 18-5, Chowringhee Road, Calcutta.

M. C. Sircar & Sons, 75, Harrison Road, Calcutta.

Higginbotham & Co., Madras.

V. Kalyanarama Iyer & Co., Madras.

G. A. Natesan & Co., Madras.

S. Murthy & Co., Madras.

Thompson & Co., Madras.

Temple & Co., Madras.

Combridge & Co., Madras.

P. R. Rama Iyer & Co., Madras.

Thacker & Co., Ltd., Bombay.

A. J. Combridge & Co., Bombay.

D. B. Taraporevala, Sons & Co., Bombay.

Radhabai Atmaram Sagoon, Bombay.

Sunder Pandurang, Bombay.

Gopal Narayan & Co., Bombay.

Ram Chandra Govind & Son, Kalbadevi, Bombay.

N. B. Mathur, Superintendent, Nazir Kanun Hind Press, Allahabad.

A. Chand & Co., Punjab.

Rai Sahib M. Gulab Singh & Sons, Mufid-i-Am Press, Lahore and Calcutta.

Superintendent, American Baptist Mission Press, Rangoon.

S. C. Talukdar, Proprietor, Students and Company, Cooch Behar.

A. M. & J. Ferguson, Colombo, Ceylon.

# A Note on the M'Fadyean Staining Reaction for Anthrax Bacilli.

BY

MAJOR J. D. E. HOLMES, M.A., D.Sc., M.R.C.V.S.

*Imperial Bacteriologist.*

In the *Journal of Tropical Veterinary Science*, Vol. IV, No. 1, pp. 68—70, Mr. Mitter of the Bengal Veterinary College drew attention to a supposed difference between the staining reactions of anthrax bacilli in that Province of India and in the Continent of Europe. In brief, he was unable to obtain the violet staining reaction which M'Fadyean described as being diagnostic of anthrax bacilli when an anthrax film is treated with a 1 per cent. methylene blue solution. In 1903, in the *Journal of Comparative Pathology and Therapeutics*, M'Fadyean published a paper in which he fully describes the method of obtaining the violet reaction, and remarks on its value as a diagnostic agent both in fresh and in putrid anthrax blood. There are many points of technique on which the author lays special stress and which should be carefully noted by everyone wishing to obtain similar results.

The reaction is obtainable only in preparations of anthrax from blood and other body fluids, not in artificial cultures of the bacilli. Schaffer of Berlin, while confirming M'Fadyean observations, found that when anthrax was grown on blood serum, on which medium the bacilli form capsules, the red staining of the capsules could be observed. The violet staining is constant in anthrax of any of the domesticated animals as well as in the mouse, guinea pig and rabbit. It is obtainable immediately after death, and it may still be detectable when all the anthrax bacilli have undergone dissolution in the unopened carcase. The reaction is diagnostic of anthrax. In a well prepared film of anthrax, the color reaction is so marked that in most instances it is possible to detect the trace of red or purple by naked eye inspection, especially when it is held up to the light.

To prepare the film, a smear preparation is made in the ordinary method. The film should not be very thin, for then the fixation is liable to be too complete. For the same reason, the film should be spread on a slide, and not on a cover glass. After drying at ordinary tempera-



ture, the slide is fixed, by lowering it, film side upwards, into the flame of a Bunsen burner, for a second. Repeat this three times, or until the under surface of the glass is a little too hot to be borne by the skin of the palm of the hand. Particular attention should be paid to the process of fixation, as the violet reaction is obtained only when the preparation is *imperfectly fixed*.

M'Fadyean draws special attention to this. "It is important to notice that what might be thought trivial departures from the directions given above will entirely prevent one from obtaining the reaction. For instance, if the film be fixed with sublimate formalin or osmic acid solutions, no trace of the violet reaction will be obtained. Failure will also occur if, in fixing the film by dry heat, the temperature is allowed to rise as high as 150°C. 100°C is sufficient, and it should not be allowed to rise much above that. Again, the film ought not to be very thin. Partly for this reason, and partly because of over-heating in the Bunsen flame, such thin films as are obtained by pressing two cover glasses together, generally fail to show the violet reaction. As an alternative method of fixation, the slides or cover glasses carrying the dried films may be immersed for a few minutes in absolute alcohol or methylated spirit. The stain must not be applied to the hot slide, or heated until the steam begins to rise, as is recommended for some methods of staining, nor must the preparation be washed in alcohol after staining."

It is also necessary to be careful that the stain is lightly washed off and that no pressure is used when drying the film between absorbent paper. The violet stained amorphous granules of the capsule, being imperfectly fixed, are easily removed from the film, and rough handling in washing or drying may cause an apparent failure to obtain the reaction. As regards the stain, M'Fadyean was, at first, of opinion that it was necessary to use a 1 per cent. solution of methylene blue which had been in stock for some time and thus acquired a polychromatic quality, or that it was necessary to add a  $\frac{1}{2}$  per cent. bicarbonate of soda to a freshly prepared 1 per cent. solution of Grubler's powder. Subsequently, it was found that freshly prepared, pure medicinal methylene blue gave the reaction, without the addition of bicarbonate of soda.

For some years past I have made use of the violet reaction in the diagnosis of anthrax, and have never failed to obtain a positive result, in anthrax blood, when the above directions have been carefully adhered to. I have used it in summer and winter temperature, both on the hills and on the plains in India, and found no variation.



Digitized by the Internet Archive  
in 2008 with funding from  
Microsoft Corporation

FIGURE 1.



- A. Staining reaction of bacilli almost completely fixed.
- B. Staining reaction of bacilli incompletely fixed.
- C. Staining reaction of bacilli and amorphous granules of the capsules when fixation has been very slight.



I am therefore inclined to believe that Mr. Mitter's failure to obtain the violet reaction in anthrax blood is due to some defect in technique. He mentions that his films were fixed by "*passing three times through the flame of a Bunsen burner.*" This would certainly cause complete fixation and a negative result. The slide should be lowered, film side up, on to the top of the flame, for a second, so that the flame should not reach the film side, but simply heat the under surface of the slide. It will generally be found that the reaction varies, in different parts of the film, in accordance with the degree of fixation. In the illustration attached, (A) shows where almost complete fixation has occurred. Consequently, the capsule of the bacilli appears as a thin violet stained line. In (B), fixation was incomplete, and the bacilli are surrounded by a thick, disintegrating violet capsule. In (C), the fixation has been very slight and the violet stained capsules are more swollen and disintegrated, and amorphous granules of the completely disintegrated capsules are seen lying apart from the bacilli.

There is no reason to suppose that the anthrax bacilli in India present any variation in virulence, staining reactions, or other biological characters, from those possessed by anthrax organisms met with in Europe or other countries.

In Europe, inoculated anthrax proves fatal to sheep, guinea pigs, rabbits, horses and cattle. In India, inoculated anthrax is equally fatal to these species of animals with the exception of cattle. When Indian cattle are inoculated with anthrax, they show a high temperature reaction, with slight local swelling at seat of inoculation, for three to four days—and recover. Very few cases have a fatal termination. Recently, through the courtesy of Sir John M'Fadyean, I obtained a culture of anthrax from his laboratory, with the object of comparing the virulence of the English and the Indian strain, in different species of animals in India. Both strains showed identical degrees of virulence. Sheep, guinea pigs and rabbits were inoculated with a like amount of a 48 hours broth culture. The mortality, and the period from time of inoculation to death, were in each species very similar. In cattle inoculated from the English strain the reaction was identical with that from the Indian virus. No mortality among cattle resulted.

# BOTANICAL SERIES.—continued.

- Vol. I, No. II. Indian Wheat Rusts by E. J. BUTLER, M.B., F.L.S.; and J. M. HAYMAN Price, Rs. 3.
- Vol. I, No. III. Fungus Diseases of Sugarcane in Bengal by E. J. BUTLER, M.B., F.L.S. Price, Rs. 3.
- Vol. I, No. IV. *Gossypium obtusifolium*, Roxburgh, by I. H. BURKILL, M.A. Price, Re. 1.
- Vol. I, No. V. An Account of the Genus *Pythium* and some *Chytridiaceæ* by E. J. BUTLER, M.B., F.L.S. Price, Rs. 4-8.
- Vol. I, No. VI. *Cephaluros virescens*, Kunze; The Red Rust of Tea by HAROLD H. MANN, D.Sc.; and C. M. HUTCHINSON, B.A. Price, Rs. 4.
- Vol. II, No. I. Some Diseases of Cereals caused by *Sclerospora Graminicola* by E. J. BUTLER, M.B., F.L.S. Price, Re. 1-8.
- Vol. II, No. II. The Indian Cottons by G. A. GAMMIE, F.L.S. Price, Rs. 7-8.
- Vol. II, No. III. Note on a Toxic Substance excreted by the Roots of Plants by F. FLETCHER, M.A., B.Sc. Price, Re. 1-8.
- Vol. II, No. IV. Studies in root Parasitism.—III.—The Haustorium of *Olaæ scandens* by C. A. BARRER, M.A., F.L.S. Price, Rs. 2-8.
- Vol. II, No. V. Studies in root Parasitism.—IV.—The Haustorium of *Cuscuta Rheedii* by C. A. BARRER, M.A., F.L.S. Price, Rs. 2-8.
- Vol. II, No. VI. Some Experiments in the Hybridising of Indian Cottons by P. F. Fyson, B.A., F.L.S. Price, Re. 1-8.
- Vol. II, No. VII. The Varietal Characters of Indian Wheats by ALBERT HOWARD, M.A., F.L.S. and GABRIELLE L. C. HOWARD, M.A. Price, Re. 1.
- Vol. II, No. VIII. The Mulberry Disease Caused by *Coryncum mori* Nom. in Kashmir, with notes on other Mulberry Diseases by E. J. BUTLER, M.B., F.L.S. Price, Re. 1-8.
- Vol. II, No. IX. The Wilt Disease of Pigeon-Pea and the parasitism of *Neocosmospora vasinfecta*, Smith, by E. J. BUTLER, M.B., F.L.S. Price, Rs. 3.
- Vol. III, No. I. Studies in Indian Tobaccos No. 1. The Types of *Nicotiana rustica*, L. Yellow Flowered Tobacco by ALBERT HOWARD, M.A., A.R.C.S., F.L.S. and GABRIELLE L. C. HOWARD, M.A. Price, Rs. 4.
- Vol. III, No. II. Studies in Indian Tobaccos No. 2. The Types of *Nicotiana tabacum*, L., by ALBERT HOWARD, M.A., A.R.C.S., F.L.S.; and GABRIELLE L. C. HOWARD, M.A. Price, Rs. 9.
- Vol. III, No. III. Studies in Indian Fibre Plants, No. 1. On two varieties of Sann, *Crotalaria juncea*, L., by ALBERT HOWARD, M.A., A.R.C.S., F.L.S.; and GABRIELLE L. C. HOWARD, M.A. Price, Re. 1.
- Vol. III, No. IV. The Influence of the Environment on the milling and baking qualities of wheat in India No. 1. The Experiments of 1907-08 and 1908-09. By ALBERT HOWARD, M.A., A.R.C.S., F.L.S.; H. M. LEAKE, M.A., F.L.S.; and GABRIELLE L. C. HOWARD, M.A. Price, Re. 1-8.
- Vol. III, No. V. The Bud Rot of Palms in India by E. J. BUTLER, M.B., F.L.S. Price, Rs. 2.
- Vol. III, No. VI. The Economic Significance of Natural Cross-fertilization in India by ALBERT HOWARD, M.A., A.R.C.S., F.L.S.; GABRIELLE L. C. HOWARD, M.A.; and ABDUR RAHMAN KHAN. Price, Rs. 4-8.
- Vol. IV, No. I. Millets of the Genus *setaria* in the Bombay Presidency and Sind by G. A. GAMMIE, F.L.S., Imperial Cotton Specialist. Price, Re. 1.
- Vol. IV, No. II. Studies in Indian Fibre Plants, No. 2, on some New Varieties of *Hibiscus cannabinus*, L., and *Hibiscus Sabdariffa*, L., by ALBERT HOWARD, M.A., A.R.C.S., F.L.S.; and GABRIELLE L. C. HOWARD, M.A. Price, Rs. 3.
- Vol. IV, No. III. Notes on the Incidence and Effect of Sterility and Cross-fertilization in the Indian Cottons by H. M. LEAKE, M.A. (Cantab.), F.L.S.; and RAM PRASAD. Price, Re. 1.
- Vol. IV, No. IV. Note on the Inheritance of Red Colour and the Regularity of Self-fertilization in the *Corchorus capsularis*, the common Jute plant, by I. H. BURKILL, M.A., and R. S. FINLOW, B.Sc., F.C.S. Price, Re. 1.
- Vol. IV, No. V. Observations on Certain Extra-Indian Asiatic Cottons by H. M. LEAKE, M.A., F.L.S.; and RAM PRASAD, Assistant to the Economic Botanist, U. P. Price, Re. 1-8.
- Vol. IV, No. VI. The Morphology and Parasitism of *Rhizoctonia* by F. J. F. SHAW, B.Sc., A.R.C.S., F.L.S. Price, Rs. 2.
- Vol. V, No. I. The Inheritance of some Characters in Wheat—I, by A. HOWARD, M.A., A.R.C.S., F.L.S.; and GABRIELLE L. C. HOWARD, M.A. Price, Re. 1.
- Vol. V, No. II. The Influence of the Environment on the Milling and Baking Qualities of Indian Wheats. No. 2—The Experiments of 1909-10 and 1910-11, by A. HOWARD, M.A., A.R.C.S., F.L.S.; GABRIELLE L. C. HOWARD, M.A.; and H. M. LEAKE, M.A., F.L.S. Price, Re. 1.



## BOTANICAL SERIES—continued.

- Vol. V, No. III. The Varieties of Soy Beans found in Bengal, Bihar and Orissa and their Commercial Possibilities, by E. J. WOODHOUSE, M.A.; and C. S. TAYLOR, B.A. Price, Rs. 2.
- Vol. V, No. IV. On *Phytophthora parasitica* nov. spec., by J. F. DASTUR, B.Sc. Price, Rs. 2.
- Vol. V, No. V. Studies in *Peronosporaceæ*, by E. J. BUTLER, M.B., F.L.S., Imperial Mycologist; and G. S. Kulkarni, L. Ag., Mycological Assistant, Bombay. Price, Rs. 2.
- Vol. V, No. I. Notes on Pollination and Cross-Fertilisation in the Common Rice Plant. *Oryza sativa*, Linn., by G. P. HECTOR, M.A., B.Sc. Price, Re. 1.
- Vol. V, No. II. A Sclerotial Disease of Rice, by F. J. F. SHAW, B.Sc. (Lond.), A.R.C.S., F.L.S. Price, Re. 1.
- Vol. VI, No. III. Inheritance of Characters in *Nicotiana tabacum*, by GABRIELLE L. C. HOWARD, M.A. (*In the press.*)
- Vol. VI, No. IV. Studies in Indian Cottons, Part I—The Vegetative Characters by H. M. LEAKE, M.A., F.L.S.; and RAM PRASAD. (*In the press.*)
- Vol. VI, No. V. The Red Rot of Sugarcane by E. J. BUTLER, M.B., F.L.S.; and A. HAFIZ KHAN. (*In the press.*)

## CHEMICAL SERIES.

- Vol. I, No. I. The Composition of Indian Rain and Dew by J. WALTER LEATHER, Ph.D., F.I.C. Price, Re. 1.
- Vol. I, No. II. The Composition of Oil Seeds of India by J. W. LEATHER, Ph.D., F.I.C. Price, Re. 1.
- Vol. I, No. III. The Pot-Culture House at the Agricultural Research Institute, Pusa, by J. W. LEATHER, Ph.D., F.I.C. Price, Rs. 3.
- Vol. I, No. IV. Experiments on the availability of Phosphates and Potash in Soils by J. W. LEATHER, Ph.D., F.I.C. Price, Re. 1-8.
- Vol. I, No. V. Construction of Drain Gauges at Pusa by M. H. ARNOTT, M.INST.C.E., with a Preface by J. W. LEATHER, Ph.D., F.I.C. Price, Rs. 3.
- Vol. I, No. VI. The Loss of Water from Soil during Dry Weather by J. WALTER LEATHER, Ph.D., F.I.C. Price, Rs. 2.
- Vol. I, No. VII. The System Water, Calcium Carbonate, Carbonic Acid by J. WALTER LEATHER, Ph.D., F.I.C. etc.; and JATINDRA NATH SEN, M.A., F.C.S. Price, Re. 1.
- Vol. I, No. VIII. Water Requirements of Crops in India by J. WALTER LEATHER, Ph.D., F.I.C. Price, Rs. 3.
- Vol. I, No. IX. The Nature of the colour of Black Cotton Soil by H. E. ANNETT, B.Sc. (Lond.), F.C.S., M.S., E.A.C. Price, Re. 1.
- Vol. I, No. X. Water Requirements of Crops in India—II, by J. WALTER LEATHER, Ph.D., F.I.C., Imperial Agricultural Chemist. Price, Rs. 2-8.
- Vol. II, No. I. The Composition of the Milk of some Breeds of Indian Cows and Buffaloes and its Variations, Part I, by A. A. MEGGITT, B.Sc. (Lond.); and H. H. MANN, D.Sc. Price, Re. 1-8.
- Vol. II, No. II. Records of Drainage in India by J. W. LEATHER, Ph.D., F.I.C. Price, Re. 1.
- Vol. II, No. III. The *Rab* System of the Rice Cultivation in Western India by H. H. MANN, D.Sc.; N. V. JOSHI, B.A., B.Sc., L.A.G.; and N. V. KANITKAR, B.A.G. Price, Re. 1.
- Vol. II, No. IV. The Composition of the Milk of some Breeds of Indian Cows and Buffaloes and its Variations, Part II, by A. A. MEGGITT, B.Sc.; and H. H. MANN, D.Sc. Price, Re. 1-8.
- Vol. II, No. V. A Contribution to the knowledge of the Black Cotton Soils of India, by W. H. HARRISON, M.Sc., Government Agricultural Chemist, Madras, and M. R. RAMASWAMI SIVAN, B.A., Chief Assistant to the Agricultural Chemist. Price, Re. 1.
- Vol. II, No. VI. The Date Sugar Industry in Bengal—an investigation into its Chemistry and Agriculture, by H. E. ANNETT, B.Sc., F.C.S., M.S.E.A.C., assisted by G. K. LELE, L.A.G.; and BHAILAL M. AMIN, B.A. Price Rs. 3.
- Vol. III, No. I. Evaporation from a plain water surface, by J. WALTER LEATHER, Ph.D., F.I.C., Price, Re. 1.
- Vol. III, No. II. Studies in the Chemistry and Physiology of the Leaves of the Betel-vine and of the Commercial Bleaching of Betel-vine Leaves by H. H. MANN, D.Sc., D. L. SAHASRABUDHE and V. G. PATWARDHAN. Price, Re. 1-8.
- Vol. III, No. III. The Gases of Swamp Rice Soils by W. H. HARRISON, M.Sc., and P. A. SUBRAMANIA AIYER, B.A. (*In the press.*)
- Vol. III, No. IV. Experimental Error in Sampling Sugarcane by J. W. LEATHER, Ph.D., F.I.C. (*In the press.*)

## ENTOMOLOGICAL SERIES.

- Vol. I, No. I. The Bombay Locust by H. M. LEFROY, M.A., F.E.S., F.Z.S. Price, Rs. 2-8.
- Vol. I, No. II. The more important Insects injurious to Indian Agriculture by H. M. LEFROY, M.A., F.E.S., F.Z.S. Price, Rs. 3.
- Vol. I, No. III. The Indian Surface Caterpillars of the Genus *Agrotis* by H. M. LEFROY, M.A., F.E.S., F.Z.S.; and C. C. GHOSH, B.A. Price, Re. 1-8.
- Vol. I, No. IV. Individual and Seasonal Variations in *Helopeltis theivora*, Waterhouse, with description of a new species of *Helopeltis* by HAROLD H. MANN, D.Sc. Price, Re. 1-8.
- Vol. I, No. V. The *Coccidæ* attacking the Tea Plant in India and Ceylon by E. E. GREEN, F.E.S.; and HAROLD H. MANN, D.Sc. Price, Re. 1.
- Vol. I, No. VI. The Mustard Sawfly by H. M. LEFROY, M.A., F.E.S., F.Z.S.; and C. C. GHOSH, B.A. Price, Re. 1.
- Vol. II, No. I. The Rice Bug by H. M. LEFROY, M.A., F.E.S., F.Z.S. Price Re. 1.
- Vol. II, No. II. Remarks on Indian Scale Insects (*Coccidæ*) by E. E. GREEN, F.E.S., F.Z.S. Price, Re. 1-8.
- Vol. II, No. III. The Red Cotton Bug by H. M. LEFROY, M.A., F.E.S., F.Z.S. Price, Re. 1.
- Vol. II, No. IV. The Castor Semi-Looper by H. M. LEFROY, M.A., F.E.S., F.Z.S. Price, Rs. 2.
- Vol. II, No. V. The Tobacco Caterpillar by H. M. LEFROY, M.A., F.E.S., F.Z.S. Price, Re. 1-8.
- Vol. II, No. VI. The Cotton Leaf-Roller by H. M. LEFROY, M.A., F.E.S., F.Z.S. Price, Re. 1-8.
- Vol. II, No. VII. Notes on Indian Scale Insects (*Coccidæ*) by H. MAXWELL-LEFROY, M.A., F.E.S., F.Z.S. Price, Re. 1-8.
- Vol. II, No. VIII. Life Histories of Indian Insects (*Coleoptera* I), by H. MAXWELL LEFROY, M.A., F.E.S., F.Z.S. Price, Rs. 2.
- Vol. II, No. IX. Life Histories of Indian Insects—II. Some Aquatic Rhynchota and Coleoptera, by D. NOWROJEE, B.A., Assistant to the Imperial Entomologist. Price, Re. 1.
- Vol. II, No. X. Life Histories of Indian Insects—III. The Rhinoceros Beetle (*Cryctes Rhinoceros*) and the Red or Palm Weevil (*Rhynchophorus Ferrugineus*) by C. C. GHOSH, B.A., Assistant to the Imperial Entomologist. Price, Rs. 2.
- Vol. III. The Food of Birds in India by C. W. MASON, M.S.E.A.C., edited by H. MAXWELL-LEFROY, M.A., F.E.S., F.Z.S. Price, Rs. 7-8.
- Vol. IV, No. I. Eri Silk by H. MAXWELL-LEFROY, M.A., F.E.S., F.Z.S., Imperial Entomologist; and C. C. GHOSH, B.A., Assistant to the Imperial Entomologist. Price, Rs. 3.
- Vol. IV, No. II. Tetriginæ (Acridiinæ in the Agricultural Research Institute, Pusa,) with descriptions of new species by J. L. HANCOCK, F.E.S. Price, Re. 1.
- Vol. IV, No. III. The Big Brown Cricket (*Brachytrypes achatinus* Stoll) by C. C. GHOSH, B.A., Assistant to the Imperial Entomologist. Price, Re. 1.
- Vol. IV, No. IV. Life Histories of Indian Insects—*Hymenoptera* by GOVIND RAM DUTT, B.A., Assistant to the Imperial Entomologist. Price, Rs. 2.
- Vol. IV, No. V. Inquiry into the Insecticidal action of some Mineral and other Compounds on Caterpillars, by H. M. LEFROY, M.A., F.E.S., F.Z.S.; and R. S. FINLOW, B.Sc., F.C.S. Price, Re. 1-8.

## BACTERIOLOGICAL SERIES.

- Vol. I, No. I. Studies in the Bacteriological Analysis of Indian Soils No. 1 by C. M. HUTCHINSON, B.A., Imperial Agricultural Bacteriologist. (*In the press.*)
- Vol. I, No. II. Rangpur Tobacco Wilt, by C. M. HUTCHINSON, B.A. Price, Rs. 2.

## VETERINARY SERIES.

- Vol. I, No. I. Anaphylaxis in the larger animals by Major J. D. E. HOLMES, M.A., D.Sc., I.C.V.D., Imperial Bacteriologist, Muktesar. Price, Rs. 2.
- Vol. I, No. II. Salvarsan in the Treatment of Surra in Horses, Dogs and Rabbits by Major J. D. E. HOLMES, M.A., D.Sc., I.C.V.D., Imperial Bacteriologist, Muktesar. Price, Re. 1-4.
- Vol. I, No. III. Some more successful Experiments on the treatment of Surra in the Camel with recommendations for Systematic treatment, by A. S. LEESE, M.R.C.V.S. Price, Re. 1.
- Vol. I, No. IV. On the Immune bodies occurring in anti-Rinderpest Serum and on the variations occurring in the serum proteins of Animals during immunisation and hyper-immunisation, by P. HARTLEY, D.Sc. (*In the press.*)
- Vol. II, No. I. Some cases of Surra treated in the field and in the laboratory during the Autumn of 1911, by Major J. D. E. HOLMES, M.A., D.Sc., I.C.V.D. Price, Re. 1. ½



# BULLETINS ISSUED BY THE AGRICULTURAL RESEARCH INSTITUTE, PUSA.

- No. 1. Notes on Cotton in Behar in 1904, by H. M. LEFROY, M.A., F.E.S., F.Z.S., Imperial Entomologist. Price As. 4 or 6d.
- No. 2. An Outbreak of Cotton Pests in the Punjab, 1905, by H. M. LEFROY, M.A., F.E.S., F.Z.S., Imperial Entomologist. Price As. 4 or 6d.
- No. 3. The Extension of Jute Cultivation in India by R. S. FINLOW, B.Sc., F.C.S., Jute Specialist to the Government of Eastern Bengal and Assam. Price, As. 12 or 1s. 6d. (*Out of print.*)
- No. 4. First Report on the Fruit Experiments at Pusa by A. HOWARD, M.A. (Cantab.), A.R.C.S. (Lond.), F.L.S., Imperial Economic Botanist. Price, As. 6 or 6d.
- No. 5. Report on Trials of the South African Locust Fungus in India by E. J. BUTLER, M.B., F.L.S., Imperial Mycologist; and H. M. LEFROY, M.A., F.E.S., F.Z.S., Imperial Entomologist. Price, As. 2 or 3d.
- No. 6. Ticks Infesting Domesticated Animals in India by C. WARBURTON, M.A., Zoologist to the Royal Agricultural Society of England. Price, As. 4 or 6d.
- No. 7. A Preliminary Account of the Biting Flies of India by H. M. LEFROY, M.A., F.E.S., F.Z.S., Imperial Entomologist. Price, Re. 1 or 1s. 6d. (*Out of print.*)
- No. 8. Official and Recommended Methods for use in Chemical Laboratories of the Departments of Agriculture in India by J. WALTER LEATHER, Ph.D., F.I.C., Imperial Agricultural Chemist. Price, As. 4 or 6d.
- No. 9. Report on Coconut Palm Disease in Travancore by E. J. BUTLER, M.B., F.L.S., Imperial Mycologist. Price, As. 6 or 6d.
- No. 10. Treatment and Observation of Crop Pests on the Pusa Farm by H. M. LEFROY, M.A., F.E.S., F.Z.S., Imperial Entomologist; and C. S. MISRA, B.A. Price, As. 6 or 7d.
- No. 11. On Flax Dodder by A. HOWARD, M.A., A.R.C.S., F.L.S., Imperial Economic Botanist. Price, As. 4 or 6d.
- No. 12. The Making and Care of Lawns in India by A. HOWARD, M.A., A.R.C.S., F.L.S., Imperial Economic Botanist. Price, As. 4 or 6d. (*Out of print.*)
- No. 13. Sugarcane at the Partabgarh Experiment Station by G. CLARKE, F.I.C., Agricultural Chemist, United Provinces; and Khan Bahadur S. M. HADI, M.B.A.C., M.B.A.S., Assistant Director of Agriculture, United Provinces. Price, As. 4 or 6d.
- No. 14. The Milling and Baking Qualities of Indian Wheats by A. HOWARD, M.A. (Cantab.), A.R.C.S. (Lond.), F.L.S., Imperial Economic Botanist; and G. L. C. HOWARD, M.A., Associate and late Fellow of Newnham College, Cambridge. Price, As. 4 or 6d.
- No. 15. Note on the Extension of Cultivation of Fibre Plants in India. Price, As. 6 or 8d.
- No. 16. Second Report on the Fruit Experiments at Pusa by A. HOWARD, M.A. (Cantab.), A.R.C.S. (Lond.), F.L.S., Imperial Economic Botanist. Price, As. 6 or 8d.
- No. 17. The Milling and Baking Qualities of Indian Wheats, No. 2, by A. HOWARD, M.A. (Cantab.), A.R.C.S. (Lond.), F.L.S., Imperial Economic Botanist; and GABRIELLE L. C. HOWARD, M.A., Associate and late Fellow of Newnham College, Cambridge. Price, As. 6 or 8d.
- No. 18. Report on the outbreak of Blister Blight on Tea in the Darjeeling District in 1908-09 by W. McRAE, M.A., B.Sc., officiating Imperial Mycologist. Price, Re. 1 or 1s. 6d.
- No. 19. List of names used in India for common insects. Compiled in the Laboratory of the Imperial Entomologist. Price As. 12 or 1s. 2d.
- No. 20. Memorandum on Indian Wheat for the British Market by Sir JAMES WILSON, K.C.S.I. Price, As. 4 or 6d.
- No. 21. Memorandum regarding Leading Eucalypts suitable for India by F. BOOTH-TUCKER, Commissioner, Salvation Army, Simla. Price, As. 4 or 5d.
- No. 22. The Milling and Baking Qualities of Indian Wheats by ALBERT HOWARD, M.A. (Cantab.), A.R.C.S. (Lond.), F.L.S., Imperial Economic Botanist; and GABRIELLE L. C. HOWARD, M.A., Personal Assistant to the Imperial Economic Botanist, Associate and late Fellow of Newnham College, Cambridge. Price, As. 7 or 8d.
- No. 23. Insecticides—Mixtures and recipes for use against insects in the field, the orchard, the garden and the house by H. M. LEFROY, M.A., F.E.S., F.Z.S. Price, As. 12 or 1s. 2d. Second Edition, revised and enlarged by T. BAINBRIDGE FLETCHER, B.N., F.E.S., F.Z.S.
- No. 24. The Indian Saltpetre Industry by DR. J. W. LEATHER, Ph.D., F.I.C., Imperial Agricultural Chemist, and JATINDRA NATH MUKERJI, B.A., B.Sc., Second Assistant to the Imperial Agricultural Chemist. Price As. 8 or 9d.
- No. 25. Report on the Flax Experiments conducted at Dooriah in 1910-11 by E. M. VANDERKROVE, Flax Expert to the Behar Planters' Association. Price, As. 6 or 7d.
- No. 26. Note on the present Position of Cotton Investigation in India by BERNARD COVENTRY, Officiating Inspector-General of Agriculture in India. Price, As. 2 or 3d.
- No. 27. Experiments on the Cultivation of Sugarcane at the Partabgarh Experimental Station, 1909-11, by G. CLARKE, F.I.C.; H. E. ANNETT, B.Sc.; SYED ZAMIN HUSSAIN, B.A. Price, As. 5 or 6d.



**BULLETINS ISSUED BY THE AGRICULTURAL RESEARCH  
INSTITUTE, PUSA—continued.**

- No. 28. The Cultivation of Lac in the plains of India by C. S. MISRA, B.A., First Assistant to the Imperial Entomologist. Price, As. 8 or 9d.
- No. 29. Directions for the cultivation of Eri Silk. Price, As. 3 or 4d.
- No. 30. Report on the Flax Experiments conducted at Doorlah during the year 1911-12 by E. M. VANDEKERKHOVE, Flax Expert to the Behar Planters' Association. Price, As. 1-6 or 2d.
- No. 31. Wheat Experiments on the Botanical Area, Cawnpur, and their bearing on Wheat Cultivation in the United Provinces, by H. MARTIN LEAKE, M.A., Economic Botanist to the Government of United Provinces, and RAM PERSHAD, Assistant to the Economic Botanist. Price, As. 3 or 4d.
- No. 32. A Note on some interesting results following the Internal Administration of Arsenic in canker and other diseases of the foot in horses, by Major J. D. E. HOLMES, M.A., D.Sc., M.R.C.V.S., Imperial Bacteriologist, Muktesar, Price, As. 2.
- No. 33. Some aspects of the Agricultural Development of Bihar, by A. HOWARD, M.A., A.R.C.S., F.L.S. Price, As. 4 or 5d.
- No. 34. Diseases of Rice, by Dr. E. J. BUTLER, M.B., F.L.S. Price, As. 8 or 9d.
- No. 35. Report on Flax Experiments conducted at Doorlah during the year 1912-13, by E. M. VANDEKERKHOVE, Flax Expert to the Bihar Planters' Association. (*In the press.*)
- No. 36. Note on the M'Fadyean Staining reaction for Anthrax Bacilli, by Major J. D. E. HOLMES, M.A., D.Sc., I.C.V.D. Price, As. 4 or 5d.
- No. 37. Notes on Experiments with Sugarcane at Sabour, by C. SOMEBS TAYLOR, B.A. (*In the press.*)
- No. 38. Disintegration of Rice Grains by means of Alkali, by F. J. WARTH, M.Sc., and D. B. DARABSETT, B.Sc. (*In the press.*)
- No. 39. Mulberry Silk, by M. N. DE, Sericulture Assistant to the Imperial Entomologist, (*In the press.*)

**BOOKS.**

- "Indian Insect Pests" by H. M. LEFROY, M.A., F.E.S., F.Z.S. Price, Re. 1-8. (*Out of print.*)
- "Indian Insect Life" by H. M. LEFROY, M.A., F.E.S., F.Z.S., and F. M. HOWLETT, B.A., pp. 786. Price, Rs. 20.
- "Wheat in India" by A. HOWARD, M.A., A.R.C.S., F.L.S.; and GABRIELLE L. C. HOWARD, M.A., pp. 288. Price, Rs. 5.
- "A Description of the Imperial Bacteriological Laboratory, Muktesar; Its Work and Products" by Major J. D. E. HOLMES, M.A., D.Sc., M.R.C.V.S. Price, As. 8.